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SHORT COMMUNICATION

# ESTABLISHMENT OF MAREK'S DISEASE LYMPHOBLASTOID CELL LINES FROM CHICKENS WITH $B^AB^K$ OF B BLOOD GROUPS

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Since Akiyama et al. first established lymphoid cell lines from a lymphoma of Marek's disease (MD) in 1973 (Akiyama et al., 1973), several lymphoma cell lines have been established (Akiyama and Kato, 1974; Powell et al., 1974; Ikuta et al., 1976; Matsuda et al., 1976; Nazerian et al., 1977; Calnek et al., 1978), and studies on MD using these cell lines have progressed rapidly. However, none of these cell lines were defined with respect to their major histocompatibility (MHC) antigen except the GBCL-1 cell line established by Calnek et al., and this has been one of serious obstacles in studies on cellular immunity in MD. This paper reports the establishment of new MD lymphoma cell lines with known MHC antigens.

White Leghorn chickens from a closed colony named "Anthony No. 11" were kindly supplied by Shirakawa Poultry Breeding Station. The colony consists entirely of chickens with the 3 types of B blood group related with MHC antigen, $B^AB^A$, $B^AB^K$ and $B^KB^K$ (Fujio, 1972). The chickens were inoculated subcutaneously with MSB-1 cells (Akiyama and Kato, 1974) at $1\times10^6$ cells/bird at 2 or 14 days of age. The MSB-1 line was originally derived from a lymphoma of a chicken inoculated with a virulent strain of MD virus (MDV) BC-1 and it produces virulent virus continuously as reported previously (Doi et al., 1976). Nutrient mixture RPMI-1640 supplemented with 20% fetal bovine serum, 100 units of penicillin and 100 $\mu$g of streptomycin per ml was used as growth medium (GM). When cultivation of lymphomas had been initiated the GM was supplemented with 10% autoserum. Primary MD lymphomas from chickens of 60 days old were used as starting material. The tissue was chopped up, passed through fine mesh sieve, and centrifuged at 1,000 rpm for 5 min. The resulting packed cells were resuspended in the same medium and cultured at 41 C in a humidified atmosphere of 5% $CO_2$ in air. The cells were cultured by two methods: the colony forming method of Hihara et al. (1974) in soft agar medium containing 0.3% acetone-

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washed Bacto-agar, and the floating culture method of Akiyama et al. (1973). In the latter method, adherent cells were removed by transferring floating cells to a new flask every day for the first 2 or 3 days.

MD lymphomas from 13 chickens were cultured by the colony forming method. Two ovarian lymphoma cultures, obtained from different chickens, began to form colonies in the soft agar medium after 7 days. These colonies were transferred to GM after 12 and 17 days of cultivation, respectively. One of them grew continuously for over 600 days and was designated as MOGA-1, whereas the other stopped growing after 110 days. Eleven lymphomas obtained from 8 chickens were cultured by the floating method. One cell line, designated as MOGA-2, was established from an ovarian lymphomas, and grew continuously for over 400 days.

The characteristics of the new cell lines of MOGA-1 and MOGA-2 are summarized and compared with those of the MSB-1 line in Table 1. The 2 line cells did not become attached to the glass but floated free in the medium as single cells, and grew logarithmically at 41 C for 4 days after subcultivation (Fig. 1).

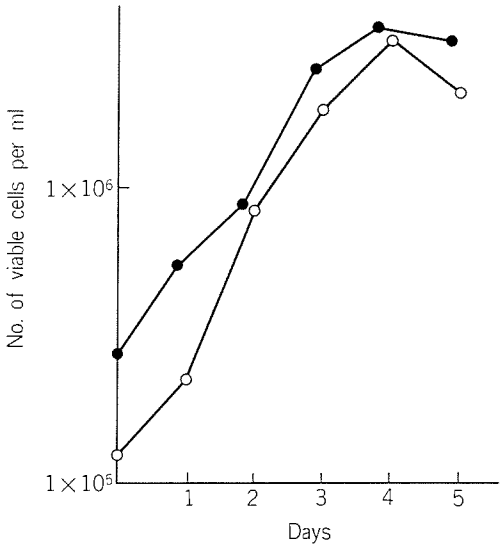



FIGURE 1. Growth curves of MOGA-1 and MOGA-2 line cells. ○, MOGA-1; ●, MOGA-2.

The doubling times of these line cells were 19 and 22 hr, respectively. Both line cells consisted mainly of round cells with a few irregular-shaped ones, and both lines were lymphoblastoid cells, as shown in Fig. 2. Electron microscopy showed that both lines had the

TABLE 1. *Characteristics of the new cell lines MOGA-1 ana MOGA-2 and of the MSB-1 line*

| Character | MOGA-1 | MOGA-2 | MSB-1 |
|---|---|---|---|
| Morphology | Lymphoblast | Lymphoblast | Lymphoblast |
| Cell volume | 513 $\mu^3$ | 586 $\mu^3$ | 589 $\mu^3$ |
| Doubling time | 19 hr | 22 hr | 10 hr |
| Cells with MATSA[a] | >95% | >95% | >95% |
| Cells with ICA[b] | 0.1-2.8% | 0.3-1.7% | 0.3-12% |
| Cells with CSA[c] | 1.5% or less | 1.1-3.2% | 3% or less |
| Herpes-type capsid | + | + | + |
| C-type particles | + | + | − |
| Karyotype | female | female | female |
| Chromosomal aberration[d] | − | − | + |
| Blood type | $B^AB^K$ | $B^AB^K$ | Not $B^A$ |

[a] Marek's disease tumor-associated surface antigen.
[b] MDV-induced intracellular antigen.
[c] MDV-induced cell surface antigen.
[d] Chromosomal aberration of No. 1 pair.

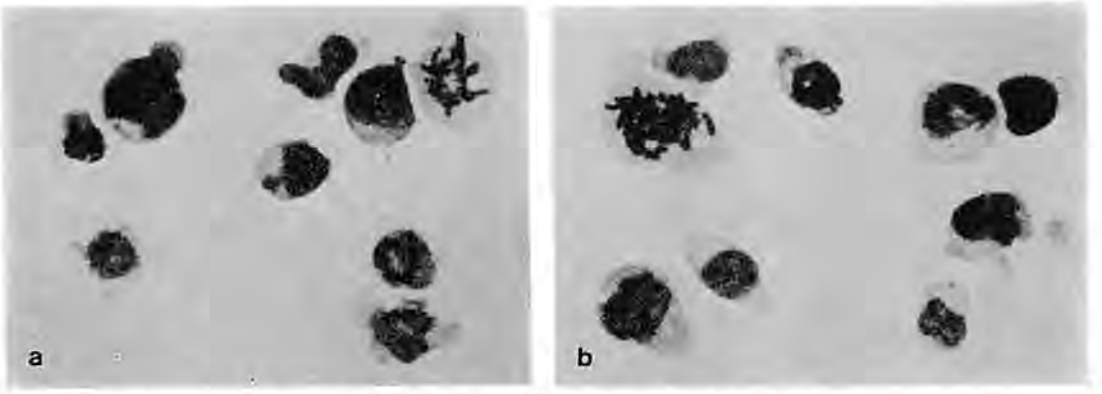


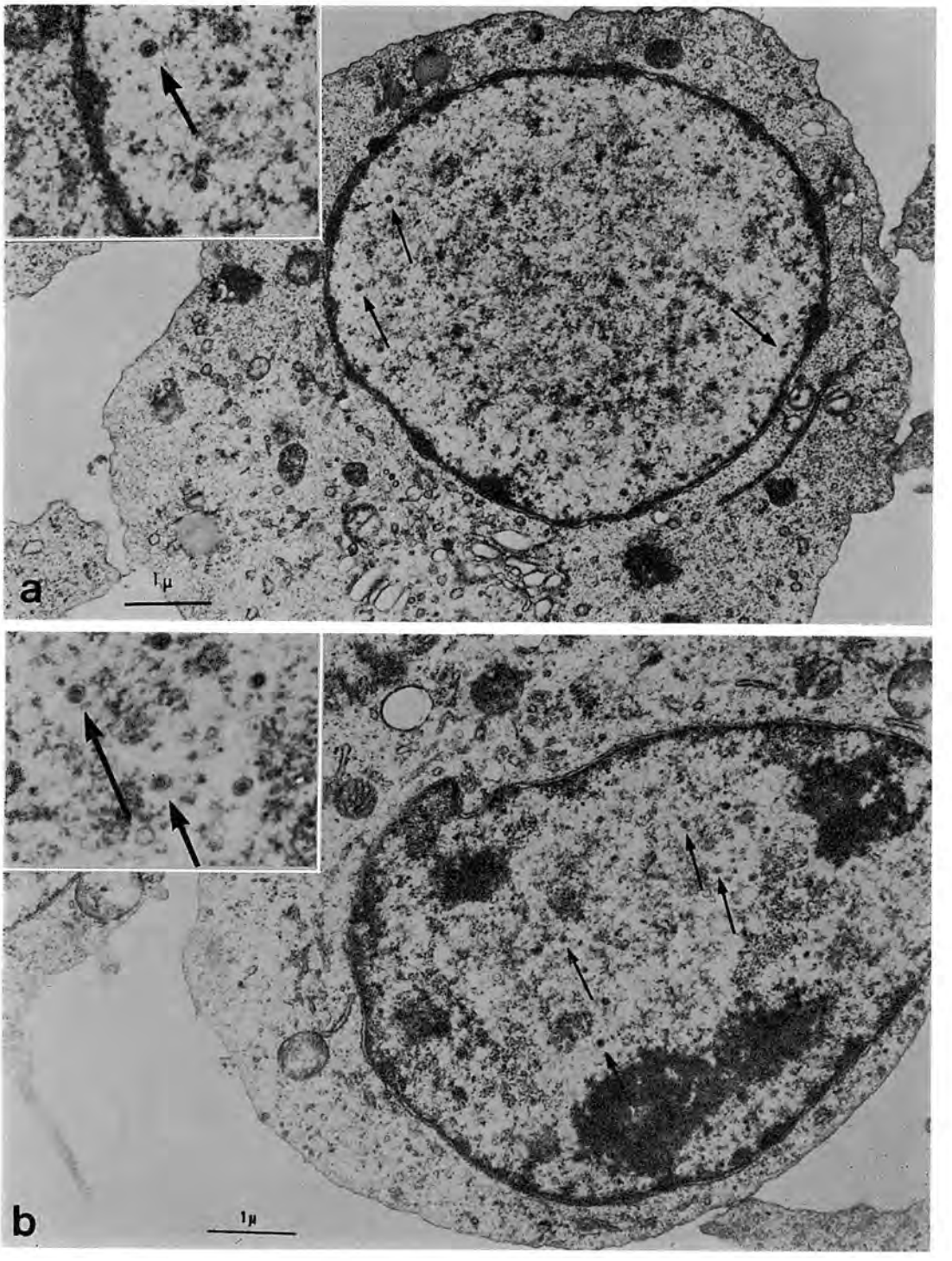

FIGURE 2. Giemsa-stained smears of lymphoblastoid line cells from Marek's disease. (a) MOGA-1; (b) MOGA-2.

typical fine structure of undifferentiated lymphocytes. The nuclei of some cells of both lines contained particles which seemed to be nucleocapsids and empty capsids of herpes type virus (Fig. 3). Some cells with herpes capsids showed degenerative changes. C-type particles were abundant, especially in extracellular spaces of both line cells. Direct immunofluorescent tests to detect MDV-induced intracellular antigen (ICA) and MDV-induced cell surface antigen (CSA) were performed by the methods of Naito et al. (1969) and Ishikawa et al. (1972), respectively. Antiserum against MD tumor-associated surface antigen (MATSA) (Powell et al., 1974; Witter et al., 1975) was prepared in rabbits by the method of Matsuda et al. (1976) and the serum obtained was absorbed several times sequentially with normal chicken blood cells, with a mixture of spleen, thymus and bursa cells, and then finally with 1104-B line cells (Hihara et al., 1974) established from lymphoid leukosis tumors induced by RNA avian leukosis virus. Living cells were used for detection of MATSA and CSA, and acetone-fixed cells for detection of ICA. A few cells of both the MOGA-1 and MOGA-2 line had ICA (0.1–2.8% and 0.3–1.7%) and CSA (less than 1.5% and 1.1–3.2%), respectively. More than 95% of the cells had MATSA (Fig. 4). MHC antigens of the line cells and tumor bearing chickens were examined by the membrane immunofluorescent test and hemagglutination test, respectively. Anti-$B^A$ and anti-$B^K$ chicken sera were kindly supplied by Dr. Baba (Laboratory of Microbiology, University of Osaka Prefecture). The MHC antigens of both cell lines and the chicken from which MOGA-2 was derived were $B^AB^K$.

Chromosomal preparations were made by the method of Owen (1965). The line cells were fixed on a slide glass by drying them over a flame after arresting mitosis by treatment with colcemid (0.1 $\mu$g/ml) for 2 hr. The dried preparations were stained with Giemsa. Karyotype analysis confirmed the female origin of both cell lines. In the MSB-1 line cells one of the short arms of No. 1 pair was much longer than the other (Akiyama and Kato, 1974; Takagi et al., 1977). However, this chromosomal aberration was not observed in MOGA-1 and MOGA-2 line cells (Fig. 5).

The MOGA-1 and MOGA-2 cell lines were clearly not the same line as MSB-1, because of their difference in MHC antigen and lack of chromosomal aberration of No. 1 pair, which is a stable marker of the MSB-1 cell line (Takagi et al., 1977). C-type particles associated with these cell lines were probably derived from the host MD chickens.

The reported rate of success in establishing MD lymphoma cell line is very low, except


a
1μ
b
1μ

◀

FIGURE 3. Thin section electron micrographs of lymphoblastoid line cells. Several herpes-type capsid structures are observed in the nucleus (arrows). (a) MOGA-1; (b) MOGA-2.

for that of Calnek et al. (1978). We employed both the colony forming method in soft agar medium and the floating culture method. Both methods were used for lymphomas from 8 chickens. One cell line was established by each method from the same lymphoma. This particular lymphoma looked immature and was probably at the progressing stage. Thus success of establishing line cells may depend upon the stage of development of the lymphoma used as source material. Autoserum probably provides good conditions for growth of lymphoma cells.

When these line cells were transplanted into syngeneic chickens in a preliminary experiment, the tumor size increased until day 11 after inoculation, and then gradually decreased. The characteristics of slow growth, expression of MATSA, avian leukosis virus-induced antigen and minor histocompatibility antigen may make it difficult to transplant these line cells into syngeneic hosts. These 2 cell lines with known major histocompatibility antigen should be useful for further immunological studies, such as transplantation experiments and in vitro cell-mediated cytotoxicity tests.

FIGURE 4. MATSA was detected on the surface of MOGA-2 line cells by membrane immunofluorescence.


FIGURE 5. No. 1 pairs of chromosomes of MOGA-1, MOGA-2, and MSB-1 line cells.

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